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T Cell Depleted Peripheral Blood Stem Cell Allotransplantation with T Cell Add Back for Patients with Hematological Malignancies: Effect of Chronic GVHD on Outcome

Aldemar Montero,[1] Bipin N. Savani,[1] Aarthi Shenoy,[1] Elizabeth J. Read,[2] Charles S. Carter,[2] Susan F. Leitman,[2] Stephan Mielke,[1] Katayoun Rezvani,[1] Richard Childs,[1] A. John Barrett[1]

[1]Stem Cell Allotransplantation Section, Hematology Branch, National Heart, Lung and Blood Institute, and [2]Department of Transfusion Medicine, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland

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Correspondence and reprint requests: A. John Barrett, MD, Stem Cell Allogeneic Transplantation Section, Hematology Branch, NHLBI, NIH, Building 10, Hatfield CRC, Room 3-5330, 10 Center Drive MSC 1202, Bethesda, MD 20892-1202 (e-mail: barrettj@nhlbi.nih.gov).

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ABSTRACT

One hundred thirty-eight patients with hematologic malignancies received myeloablative T cell-depleted peripheral blood stem cell transplant (PBSCT) from an HLA-identical sibling donor. The T cell dose was adjusted to 0.2-1 × 10^5 $CD3^+$ cells/kg. The CD34 dose was 2.7-16 × 10^6/kg. Patients with acute graft-versus-host disease (GVHD) grade <2 received 1 or 2 donor lymphocyte infusions of 10^7 $CD3^+$ cells/kg between days 45 and 100. Patients were designated according to relapse probability as standard or high relapse risk (77 and 61, respectively). Overall survival (OS), relapse-free survival, relapse, and transplant-related mortality (TRM) were 58%, 46%, 40%, and 20%, respectively, after a median follow-up of 4 years. Fifty-three (39%) and 21 (15%) patients developed grade 2-4 and 3-4 acute GVHD. Forty-two (36%) had limited and 29 (25%) had extensive chronic GVHD. In multivariate analysis, disease risk was an independent factor for OS and relapse, day-30 lymphocyte count for OS and TRM, and chronic GVHD for OS and relapse. PBSCT with early T cell add back leads to comparable rates of chronic GVHD compared with T cell-replete PBSCT. However, this chronic GVHD after T cell add back is associated with less mortality and retains a protective effect in terms of relapse, at least in the standard-risk patients.

KEY WORDS

T cell depleted • Myeloablative • Peripheral blood stem cell transplantation

INTRODUCTION

In the past decade, peripheral blood stem cell transplantation (PBSCT) has been increasingly used as an alternative to bone marrow transplantation (BMT). One potential benefit of PBSCT is the higher CD34 cell dose, about twice as high as that of marrow [1]. High stem cell doses are associated with less leukemic relapse, and lower relapse rates have been reported in some comparisons of PBSCT with BMT [2,3]. However, PBSCT also confers a higher risk of chronic graft-versus-host disease (GVHD) and possibly acute GVHD [1]. This increased risk of GVHD may be attributable to the higher doses of T cells, about a log increase over BMT. It has long been apparent that T cell depletion of BMT reduces the incidence and severity of GVHD [4,5] and transplant-related mortality (TRM). However, the increased risk of leukemic relapse from a weaker graft-versus-leukemia (GVL) effect usually results in disease-free survivals (DFSs) similar to those with BMT that are not T cell depleted (TCD) [6]. There has been relatively little experience with TCD-PBSCT partly due to the difficulty of eliminating the larger numbers of T cells in

PBSCT compared with BMT. Given the differences in the cellular composition of PBSCT and BMT, it thus remains an open question as to whether outcome from PBSCT could be improved by T cell depletion.

To explore the potential benefit of high CD34 dose PBSCT with controlled T cell dosing, we developed a protocol whereby a granulocyte colony-stimulating factor-mobilized PBSC product underwent a 4-5 log reduction of T cells before infusion. The transplantation product contained a fixed low T cell dose and a relatively high CD34 cell dose. To minimize GVHD but boost GVL effects, patients who had not developed GVHD in the first 6-8 weeks after transplantation received donor lymphocyte infusions (DLIs) at 45-100 days after transplantation. We describe outcomes in 138 consecutive patients who underwent such TCD-PBSCT and examine factors affecting relapse and survival.

METHODS

Patients

Between 1997 and 2004, patients with hematologic malignancies were treated at the National Institutes of Health with TCD-PBSCT derived from HLA-matched related donors, under consecutive protocols 97-H-0099, 99-H-0046, 02-H-0111, and 04-H-0112 approved by the National Heart, Lung and Blood Institute institutional review board (Table 1). Several patients with HLA-mismatched related donors were enrolled in these protocols but were excluded from this analysis. Written informed consent was obtained according to principles outlined in the Declaration of Helsinki.

Preparative Regimen and Immunosuppression

All patients were conditioned with total body irradiation (TBI) and cyclophosphamide 120 mg/kg (60 mg/kg per day on days −3 to −2). TBI was given twice daily in 8 equal fractions of 150-170 cGy each, from day −7 to −4. The last 56 patients also received fludarabine 125 mg/m (25 mg/m daily on days −8 to −4) in an attempt to minimize graft rejection. Cyclosporine (CSA) was the sole agent used as GVHD prophylaxis. In attempts to minimize the dose and duration of immunosuppression, 3 different schedules were evaluated: CSA was started on day −4 (first 3 cohorts), day +44 (fourth cohort), and day −6 (fifth cohort) and tapered after day +180 unless chronic GVHD occurred. In addition, in the fifth cohort, CSA was suspended between days 21 and 59. The rationale for modifying the CSA schedule and transplanted T cell dose was to find a way to reduce or avoid CSA immunosuppression after a TCD-PBSCT without causing severe acute GVHD immediately after transplantation. Cohort 1 established that some patients developed acute GVHD despite TCD to 1×10^5/kg and standard-dose CSA (target level, 200-400 μg/L). In cohorts 2 and 3 we tested the requirement for CSA to prevent acute GVHD by using a lower transplant T cell dose of 0.5×10^5/kg. CSA was reduced to a low dose (target level, 100-200 μg/L) in cohort 2 and was withheld until DLI in cohort 3. In cohort 3, 2 patients demonstrated transplant rejection [7]. Therefore, it was deemed necessary to include CSA in the peritransplantation period. Thus in cohort 4, we reduced the T cell dose further (to reduce the risk of acute GVHD) and administered low-dose CSA (to prevent graft rejection). This resulted in less acute GVHD and no graft failure. In cohort 5, we then tested the effect of stopping low-dose CSA on day 21 after transplantation.

Individual cohorts are presented in Table 1.

Stem Cell and Lymphocyte Collection

Stem cells and lymphocytes were collected from the donor in separate apheresis procedures by using a CS3000 Plus automated blood cell separator (Baxter Healthcare, Deerfield, Ill). Before stem cell mobiliza-

Table 1. *Protocol Descriptions and Outcomes*

Cohort	n	TBI (cGy)	Flu (125 mg/m²)	CD3 Dose (×10⁵/kg)	CSA Dose	DLI Dose (×10⁷/kg per day) 45	60	100*	Outcomes, n (%)§ HR	OS	RFS	Relapse	TRM
1	35	1360	—	1	SD	1		5	16 (47)	18 (53 ± 9)	13 (38 ± 8)	14 (48 ± 10)	6 (20 ± 7)
2	22	1360	—	0.5	LD	1		5	12 (55)	10 (45 ± 11)	8 (36 ± 10)	10 (53 ± 12)	4 (21 ± 9)
3	26	1200	—	0.5	LD†	1		5	9 (35)	15 (58 ± 10)	13 (50 ± 10)	6 (29 ± 10)	7 (27 ± 9)
4	35	1200	Yes	0.2	LD†	1		2	11 (31)	24 (66 ± 9)	22 (61 ± 9)	7 (22 ± 7)	6 (21 ± 8)
5	21	1200	Yes	0.2	LD‡		1		13 (62)	17 (78 ± 10)	14 (63 ± 11)	5 (26 ± 10)	2 (13 ± 9)

TBI indicates total body irradiation; Flu, fludarabine; CSA, cyclosporine; DLI, donor lymphocyte infusion; SD, standard dose (200-400 μg/L); LD, low dose (100-200 μg/L); HR, high risk; OS, overall survival; RFS, relapse-free survival; TRM, transplant-related mortality.

*Not given in patients with acute graft-versus-host disease grade ≥2 or patients in first chronic-phase-chronic myeloid leukemia (CP-CML).

†Starting day before DLI.

‡Stopped from day +21 to +59 unless graft-versus-host disease developed.

§Nonsignificant difference between cohorts (percentage listed is actuarial data).

tion, mononuclear cells were collected by a single 10- to 15-L leukapheresis and cryopreserved in liquid nitrogen in aliquots for future DLIs. Lymphocytes were usually collected from donors in a single apheresis of 10-15 L and cryopreserved in liquid nitrogen until use. Donors then received granulocyte colony-stimulating factor (filgrastim, Amgen, Thousand Oaks, Calif), 10 μg/kg per day subcutaneously for 6 days. On days 5 and 6, a 15- to 25-L leukapheresis was performed using the CS3000 Plus to obtain peripheral blood stem cells. Prophylactic calcium chloride infusions were routinely administered to avoid citrate toxicity. The minimal target CD34 dose after TCD was 3×10^6/kg.

T Cell Depletion

CD34 selection and TCD were performed by 1 of 2 selection methods. In the first cohort of patients (protocol 97-H-0099), the Ceprate selection system (CellPro, Bothell, Wash), based on biotin-avidin immunoadsorption, was used. This process included an initial $CD34^+$ selection on the Ceprate SC column, followed by a $CD2^-$ selection on a second column. After this method became unavailable, cell products were depleted of T cells with the Isolex 300i immunomagnetic cell selection system (Baxter Healthcare) using the program for simultaneous positive and negative selections. On the Isolex system, we used the manufacturer's antibody for positive selection of $CD34^+$ cells and a cocktail of 3 T cell-specific monoclonal antibodies (CD2, CD6, and CD7; a gift from Dr Ronald Gress) for negative selection of T cells. The final T cell dose of the allograft was fixed between 0.2 and 1×10^5 $CD3^+$ cells/kg depending on the protocol (Table 1) by supplementing the final product with T cells from the original unmanipulated peripheral blood stem cell component. TCD-PBSCT products were infused into patients immediately or cryopreserved in a controlled-rate freezer and stored in liquid nitrogen for subsequent thawing and infusion.

Post-transplantation Donor Lymphocyte Infusions

To prevent relapse and facilitate immune reconstitution, 1 or 2 lymphocyte infusions were scheduled between days + 45 and +100 or specifically on day +60 (Table 1), excluding patients receiving steroid treatment for acute GVHD grade ≥2.

Infection Prophylaxis

Bactrim double strength, 3 times a week was given for *Pneumocystis* prophylaxis after engraftment and continued until immunosuppression was discontinued. Patients received fluconazole during the first 100 days and high-dose acyclovir, if the patient or donor was seropositive for cytomegalovirus. Weekly cytomegalovirus monitoring (initially using blood antigenemia and later by polymerase chain reaction) was used to monitor reactivation. Valganciclovir, ganciclovir, or foscarnet was used to treat cytomegalovirus reactivation.

Definitions

To evaluate factors that affect outcome, patients were assigned to 2 risk categories. Those with acute leukemia in first remission, chronic myeloid leukemia (CML) in first chronic phase, and myelodysplastic syndrome with refractory anemia were designated standard risk (SR). Patients with more advanced disease were designated as high risk (HR). Chronic phase CML was distinguished from advanced phase (accelerated and blastic phase) according to criteria from the International Bone Marrow Transplantation Registry (IBMTR) [8]. Patients with other diagnoses (multiple myeloma, chronic lymphocytic leukemia, non-Hodgkin lymphoma, and chronic myelomonocytic leukemia) were categorized as SR or HR depending on refractoriness to prior chemotherapy or unfavorable cytogenetics. At day +30 after transplantation, the lymphocyte count (LC30, defined as absolute lymphocyte count on day 30 after transplantation) was recorded from the routine complete blood cell count. Overall survival (OS) was defined as time from transplantation until death from any cause. TRM was defined as time from transplantation until death from infectious cause, graft failure, GVHD, or secondary malignancies.

Statistical Methods

Summary statistics, such as proportions, means, standard deviations, 95% confidence intervals, medians, and ranges, were used to describe patient characteristics, pretransplantation variables, and post-transplantation outcomes. Kaplan-Meier estimates and Cox proportional hazard models were used to estimate the time-to-event distributions of OS, relapse, and TRM. Statistical associations between pretransplantation variables were investigated using correlation analysis, including Pearson correlation coefficients and Spearman rank correlation coefficients, and multiple regression analysis. Statistical tests were based on *t* tests. Chi-square tests and F tests were used to evaluate the statistical significance of covariates in multiple regression models or the Cox proportional hazard models. The Wald score and likelihood ratio tests were used to evaluate the fitness of the Cox proportional hazard models. Data analysis was performed with SPSS 13 for Windows (SPSS, Inc, Chicago, Ill).

RESULTS

Between 1997 and 2004, 148 patients were enrolled. Ten were excluded from analysis because of donor-recipient HLA mismatch. Distribution of patients by treatment cohorts is presented in Table 1. There was no difference in proportions of HR versus

Table 2. *Characteristics of Patients (n = 138)*

Age (y), median (range)	**34 (10-56)**
Male (%)	**54**
Female/male (%)	**24**
CMV seropositivity (%)	**80**
Standard risk	**77**
AML CR1	**17**
ALL CR1	**5**
CML CP1	**42**
MDS RA	**10**
Other	**3**
High risk	**61**
AML > CR1	**21**
ALL > CR1	**16**
CML AP, BP	**12**
MDS > RA	**8**
Other	**4**

CMV indicates cytomegalovirus; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; CR1, first complete remission; CP1, first chronic phase; MDS, myelodysplastic syndrome; RA, refractory anemia; AP, accelerated phase; BP, blastic phase.

SR patients between cohorts nor did their outcome differ (Table 1). Characteristics of patients are presented in Table 2. Median dose of $CD34^+$ cells $\times$ 10^6/kg was 5.8 (range, 2.7-16). One hundred twelve patients received DLI (1 DLI in 70, 2 DLIs in 42). Twenty six patients did not receive DLI.

Engraftment

Neutrophil recovery to $>0.5 \times 10^9$/L was achieved between days 10 and 18. Two patients had primary graft failure and died at days 103 and 222. Details of the kinetics of myeloid and T cell engraftment in a subset population of this study has previously been published [9].

Graft-versus-Host Disease

Excluding the 2 patients with graft failure, 83 patients (61%) had 0 or grade 1 acute GVHD. Frequencies of grade 2-4 and 3-4 GVHD were 53 (39%) and 21 (15%), respectively. Four patients with grade 3-4 acute GVHD were refractory to treatment with corticosteroids. Surprisingly, despite aggressive TCD, 15 patients (11% of all patients and 27% of patients with GVHD grade $\geq$2), including 3 of 4 steroid refractory patients, developed GVHD grade $\geq$2 before administration of DLI and did not receive DLI. The remaining 11 patients did not receive DLI because of early death (n = 7) or because they had CML in molecular remission (n = 4). There was no difference in the rate of acute GVHD between TBI doses of 1360 and 1200 cGy. Chronic GVHD was analyzed in 116 patients surviving >150 days. The 150-day landmark, rather than the conventional 100-day landmark, was chosen because most patients had an extended risk of acute GVHD from the delayed T cell add back. Of

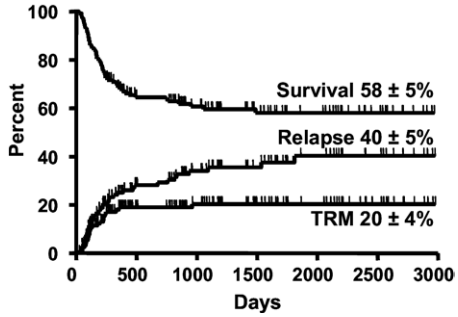

Figure 1. Outcomes after TCD-PBSCT.

116 patients at risk, 45 (39%), 42 (36%), and 29 (25%) had absent, limited, and extensive chronic GVHD, respectively. Most chronic GVHD responded well to a regimen of alternate-day prednisone and CSA and the disease persisted only in a minority of patients. As a consequence, there was no late TRM attributable to chronic GVHD (Figure 1). Of the 26 patients who did not receive DLI, 11 survived beyond day 150, and 6 (55%) developed chronic GVHD.

Forty-four of 68 patients with an LC30 above the median of 0.3×10^9/L and 28 of 68 patients with an LC30 below the median developed chronic GVHD (chi-square, 6.53; df, 1; $P = .006$). The dose of $CD34^+$ cells was not correlated with development of acute (chi-square, 0.031; df, 1; $P = .86$) or chronic (chi-square, 0.46; df, 1; $P = .49$) GVHD. Other variables that did not correlate with the development of GVHD were age, entered as a categorical variable below or above the median, dose and timing of CSA, DLI (yes/no), female versus male sex, or use of fludarabine.

Transplantation Outcome

Eighty four patients were alive after a median follow-up of 47 months (range, 8.5-99). Forty-two patients relapsed at a median of 166 days after transplantation (range, 29-1805). Fifty-four patients died, with leukemia relapse being the major cause of mor-

Table 3. *Causes of Death after T Cell Depleted Peripheral Blood Allotransplantation*

Causes of Death	n (%)
Relapse	**28 (20)**
TRM	**25 (18)**
Infection	**8**
GVHD/infection*	**7**
IPS/ARDS	**6**
Graft failure	**2**
Second graft failure	**2**
Accident	**1**
Total	**54 (39)**

TRM indicates transplant-related mortality; GVHD, graft-versus-host disease; IPS, idiopathic pneumonia syndrome; ARDS, acute respiratory distress syndrome.

*After immunosuppression for GVHD.

tality, followed by TRM (Table 3). Twenty-five patients died of TRM at a median of 108 days (range, 23-953), with only 1 patient dying >1 year after transplantation. Actuarial survival, relapse-free survival, relapse, and TRM were 58 ± 5%, 46 ± 5%, 40 ± 5%, and 20 ± 4%, respectively (Figure 1).

Variables Affecting Outcomes

Univariate analysis. Factors entered in univariate analysis for survival are listed in Table 4. Compared with those with SR disease, patients allocated to the HR category had worse survival (35% versus 76%; $P < .0001$) and DFS (28% versus 59%; $P < .0001$), more relapse (58% versus 29%; $P < .0001$), and higher incidence of TRM (28% versus 14%; $P = .02$). Patients with LC30 above the median had better survival (73% versus 44%; $P = .0006$) and DFS (63% versus 31%; $P = .001$), lower TRM (9% versus 32%; $P = .0007$; Figure 2), and a tendency toward a low incidence of relapse (30% versus 50%; $P = .1$). The presence of chronic GVHD was found to be strong prognostic factor for survival and DFS, which was clearly related to decreased rates of relapse (Figure 3).

Multivariate analysis. Factors affecting outcomes with $P \leq .1$ in the univariate analysis were entered into a multivariate analysis. Risk category, chronic GVHD,

Table 4. *Univariate Analysis (Log Rank) of Factors Affecting Survival*

Variable	OS	
	%	*P*
Age (median 34)		0.34
Below median	64 ± 6	
Above median	54 ± 6	
CD34 dose (median 5.8 × 10^6/kg)		0.96
Below	57 ± 7	
Above	60 ± 6	
Gender		0.83
Female (62)	58 ± 7	
Male (76)	59 ± 6	
Donor-patient sex match		0.27
Female to male (33)	59 ± 5	
Others (105)	58 ± 9	
DLI* (n)		0.65
1 (70)	54 ± 10	
2 (46)	46 ± 9	
CSA dose (n)		0.18
Standard (34)	53 ± 9	
Low (22)	45 ± 11	
Late or interrupted (82)	65 ± 6	
Acute GVHD (n)		0.94
Absent (64)	58 ± 7	
Present (74)	58 ± 6	
Chronic GVHD† (n)		<.0001
Absent (45)	46 ± 9	
Present (71)	83 ± 5	
Risk status		<.0001
Standard (77)	76 ± 5	
High (61)	35 ± 7	
LC30 (median 609 000/μL)		.0006
Below	44 ± 6	
Above	73 ± 6	

OS indicates overall survival; DLI, donor lymphocyte infusion; CSA, cyclosporine; GVHD, graft-versus-host disease; LC30, absolute lymphocyte count on day 30 after transplantation.
*Patients without DLI or with CML are excluded.
†Landmark analysis for survivors beyond +150 days.

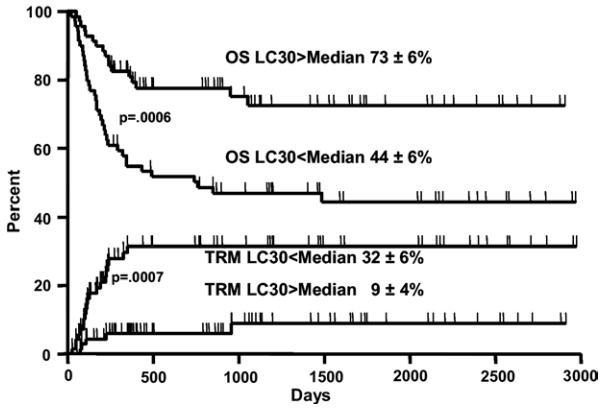

Figure 2. Patients with LC30 above the median had significantly better survival and lower TRM.

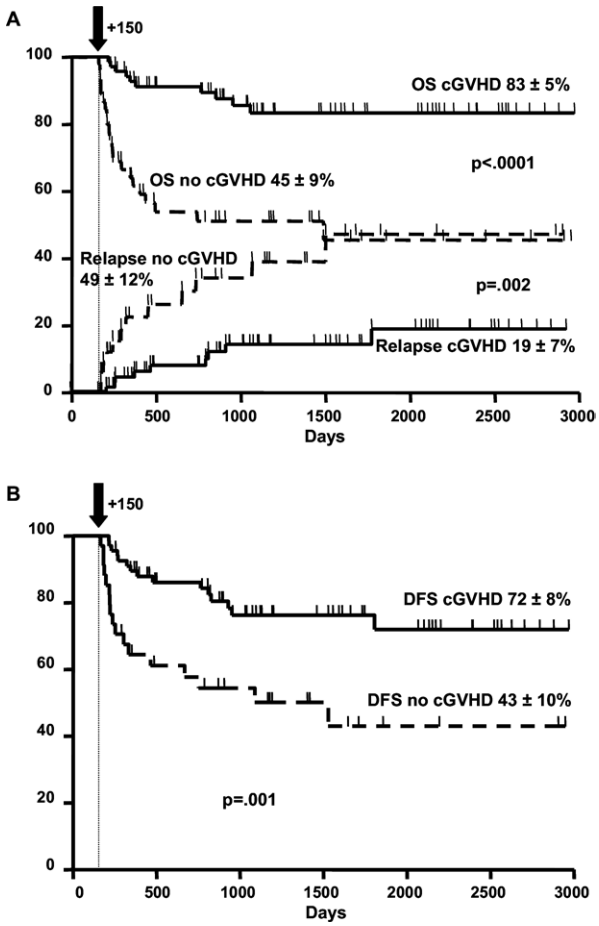

Figure 3. Landmark analysis of patients surviving beyond day +150. Chronic GVHD (cGVHD) exerts its beneficial effects by decreasing the rate of relapse. A, OS; B, DFS.

Table 5. *Multivariate Analysis: Factors Associated with Transplantation Outcome*

Outcome	Variable	RR	95% CI	P
OS	cGVHD	5.6	2.8-11.1	<.0001
	Median ≥LC30	2.2	1.2-4.0	.008
	High risk	0.3	0.1-0.5	<.0001
Relapse	No cGVHD	4.6	2.3-9.0	<.0001
	Standard risk	0.3	0.2-0.6	<.0001
TRM	Median ≤LC30	4.9	1.8-13.1	.002
	Standard risk	0.4	0.2-0.9	.018
DFS	cGVHD	5.7	2.5-12.9	<.0001
	High risk	0.3	0.1-0.6	.002
	Median ≥LC30	3.5	1.5-8.0	.003

RR indicates risk ratio; CI, confidence interval; OS, overall survival; cGVHD, chronic graft-versus-host disease; LC30, absolute lymphocyte count on day 30 after transplantation; TRM, transplant-related mortality; DFS, disease-free survival.

and LC30 were independent factors affecting survival and DFS. However, only the presence of chronic GVHD and SR disease were independently associated with a reduction in disease relapse, and LC30 above the median and SR disease were independently associated with reduction in TRM (Table 5). In a landmark analysis of patients who were alive and free of disease after 150 days, disease risk no longer maintained significance as an independent factor affecting DFS.

Interrelation between variables. $CD34^+$ cell dose did not influence OS or chronic GVHD incidence. However, patients with a $CD34^+$ cell dose above the median had a higher likelihood of having an LC30 above the median (chi-square, 6.5; df, 1; $P = .017$), and patients with an LC30 above the median had more chronic GVHD and better survival.

DISCUSSION

Although stem cell transplantation using myeloablative TBI-based conditioning is an effective and time-tested transplantation technique for hematologic malignancies [10], success is limited by GVHD and relapse, especially in HR leukemias. For over a decade we have sought to define the cellular components of the transplantation and GVHD prophylaxis regimen that produce the best outcome after TBI-based myeloablative stem cell transplantation. In a series of transplantation protocols using different TCD approaches, we identified CD34 cell doses $>3 \times 10^6$/kg as a critical factor for success [11,12]. We used PBSCT because of its potential to optimize transplantation outcome through the high CD34 doses available. Even after TCD, with the 2 apheresis collections we were able to achieve a median target CD34 cell dose identical to the dose range reported to provide a measure of protection against relapse [12]. As better depletion techniques became available, the transplantation T lymphocyte dose was progressively reduced. However, it is surprising that TCD was not effective at reducing the incidence of acute or chronic GVHD. Without CSA after transplantation, a T cell dose of 5×10^4/kg was associated with unacceptable rates of acute GVHD before DLI [7]. In subsequent protocols, the T cell dose was reduced to 2×10^4/kg, which still caused acute GVHD before DLI, even in the presence of CSA. Acute GVHD (grade ≥2) before DLI occurred mostly in cohort 5 patients (n = 9) whose CSA was stopped on day 21 despite the low transplanted T cell dose of 2×10^4/kg. Thus, the goal of delivering a TCD-PBSCT without immunosuppression after transplantation has not been achieved and low-dose CSA remains an important component of treatment after transplantation. These results suggest that PBSCT lymphocytes have a greater potential to cause GVHD than those from bone marrow.

These protocols resulted in a total incidence of limited and extensive chronic GVHD of around 65%, which is similar to that reported for unmanipulated PBSCT. However, the morbidity encountered in our patients was low and no patient died of chronic GVHD or associated late effects of chronic GVHD. This may have been attributable to the TCD and supports our earlier observation that low T cell dose at transplantation and not the later DLI is the major factor determining chronic GVHD disease and severity [13]. In this study, we showed that chronic GVHD is a strong predictor of survival due mostly to the decreased risk of relapse, similar to that described in unmanipulated PBSCT. Our results contrast with a large IBMTR study that did not show decreased relapse with the development of chronic GVHD in TCD-BMT [14]. The difference in outcomes between these 2 studies of TCD stem cell transplantation could be due to the use of DLI in our protocol. Our study showed a higher incidence of chronic GVHD compared with that reported after BMT [1,15]. We changed the schedule of giving donor lymphocytes as a preemptive approach to preventing relapse. The delayed add back of lymphocytes to selected patients without GVHD appeared to be safe and the overall mortality from acute GVHD or acute GVHD and infection was very low (n = 7, or 5%), with 4 patients dying from GVHD before receiving DLI and 3 dying from GVHD after DLI. The design of these trials limited our ability to determine if the second DLI was beneficial: patients with CML in first chronic phase (ie, SR patients) were scheduled for a single DLI, which may have biased the results of a single DLI toward a better outcome. Even after excluding patients with CML, any benefit from the second DLI was confounded by competing variables: (1) occurrence of acute GVHD grade ≥2, which excluded patients from receiving DLI; and (2) death before a second DLI. In several analyses, we did not identify a clear benefit from any particular strategy for delayed

lymphocyte add back [11,13,16] and found that variations in the schedule and dose of donor lymphocytes did not emerge as factors affecting outcome. Thus, the role of preemptive DLI remains unclear.

Overall, the outcomes for this transplantation approach were closely comparable to those achieved with myeloablative stem cell transplantation in similar patient populations. As is generally observed, patients with advanced disease had a high risk of relapse and mortality. However, patients with earlier stages of leukemia had excellent outcomes, comparing favorably to other PBSCT approaches.

The relatively large cohort and long median follow-up of 4 years allowed us to search for factors that affected outcome. After disease risk, chronic GVHD was the most powerful determinant of survival, DFS, and relapse. Our results emphasize the importance of chronic GVHD in sustaining a GVL effect, whereas the low mortality in patients with chronic GVHD translated into a survival advantage. Thus, it appears that the transplantation approach with TCD-PBSCT achieved a compromise between the deleterious effects of chronic GVHD and its beneficial antileukemic effect. However, it should be noted that patients with HR disease are more likely than those with SR disease to relapse before the development of chronic GVHD and, as such, benefit less from a protective effect of chronic GVHD.

An important early predictor of TRM and survival was the LC30. This observation extends our previous analysis and is consistent with other studies suggesting that early lymphocyte recovery is favorable for outcome [17,18]. In a previous study we showed that LC30 was an independent prognostic factor for transplantation outcome in patients with CML [19], suggesting that it may be possible to control lymphocyte recovery by optimizing CD34 dose. Reports on the prognostic value of $CD34^+$ cell dose on outcome differ. In a large IBMTR analysis in BMT and PBSCT, higher CD34 cell doses were associated with less treatment failure [20]. In contrast, Urbano-Ispizua et al [21] who studied PBSCT found that a higher CD34 dose was associated with worse survival due to higher TRM. Several other reports have described the implications of $CD34^+$ cell dose on acute and chronic GVHD, relapse, and survival in T cell replete or nonmyeloablative stem cell transplantation [10,22-25]. In our study, CD34 dose did not predict for survival, relapse, TRM, or rates of GVHD, perhaps because the dose range fell within an optimum zone for outcome, and cell doses universally exceeded a critical minimum level.

In summary, our results emphasize the importance of chronic GVHD for sustaining a GVL effect after allogeneic stem cell transplantation and show that it is possible to design protocols that minimize morbidity and mortality from chronic GVHD. The relatively favorable effect of chronic GVHD was probably due to the relatively short duration and mild manifestation of the disease even when extensive. It is disappointing, however, that patients with HR leukemia continue to relapse despite chronic GVHD. Further, attempts to intensify GVL effects have been thwarted by our inability to safely eliminate immunosuppression after transplantation. Thus, further modifications to the strategy of TCD-PBSCT appear unlikely to improve the outcome for patients with HR leukemia.

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